Neuropsychopharmacology (2006) 31, 1659–1674

www.neuropsychopharmacology.org

Chronic Lithium Chloride Administration Attenuates Brain NMDA Receptor-Initiated Signaling via Arachidonic Acid in Unanesthetized Rats

Mireille Basselin*[,1], Lisa Chang[1], Jane M Bell[1] and Stanley I Rapoport[1]

[1]*Brain Physiology and Metabolism Section, National Institute on Aging, National Institutes of Health, Bethesda, MD, USA*

It has been proposed that lithium is effective in bipolar disorder (BD) by inhibiting glutamatergic neurotransmission, particularly via *N*-methyl-D-aspartate receptors (NMDARs). To test this hypothesis and to see if the neurotransmission could involve the NMDAR-mediated activation of phospholipase A_2 (PLA_2), to release arachidonic acid (AA) from membrane phospholipid, we administered subconvulsant doses of NMDA to unanesthetized rats fed a chronic control or LiCl diet. We used quantitative autoradiography following the intravenous injection of radiolabeled AA to measure regional brain incorporation coefficients k^* for AA, which reflect receptor-mediated activation of PLA_2. In control diet rats, NMDA (25 and 50 mg/kg i.p.) compared with i.p. saline increased k^* significantly in 49 and 67 regions, respectively, of the 83 brain regions examined. The regions affected were those with reported NMDARs, including the neocortex, hippocampus, caudate-putamen, thalamus, substantia nigra, and nucleus accumbens. The increases could be blocked by pretreatment with the specific noncompetitive NMDA antagonist MK-801 ((5R,10S)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d] cyclohepten-5,10-imine hydrogen maleate) (0.3 mg/kg i.p.), as well by a 6-week LiCl diet sufficient to produce plasma and brain lithium concentrations known to be effective in BD. MK-801 alone reduced baseline values for k^* in many brain regions. The results show that it is possible to image NMDA signaling via PLA_2 activation and AA release *in vivo*, and that chronic lithium blocks this signaling, consistent with its suggested mechanism of action in BD.
Neuropsychopharmacology (2006) **31,** 1659–1674. doi:10.1038/sj.npp.1300920; published online 9 November 2005

Keywords: arachidonic acid; lithium; bipolar disorder; phospholipase A_2; MK-801; NMDA

INTRODUCTION

Lithium (Li^+) has been used to treat bipolar disorder (BD) for about 50 years, but its mechanism of action is not agreed on (Barchas *et al*, 1994; Cade, 1999). One suggestion has been that it acts in part by inhibiting glutamatergic neurotransmission, particularly via *N*-methyl-D-aspartate (NMDA) receptors (NMDARs) (Bauer *et al*, 2003; Farber *et al*, 2002; Krystal *et al*, 2002; Stewart and Reid, 2002; Zarate *et al*, 2003). Supporting this is evidence of disturbed markers of NMDA functioning in the BD brain (Itokawa *et al*, 2003; Mundo *et al*, 2003; Scarr *et al*, 2003), and data that drugs effective against BD, such as lamotrigine, carbamazepine and valproic acid, have direct or indirect NMDA antagonist properties (Farber *et al*, 2002; Hough *et al*, 1996; Kubota *et al*, 1994; McIntyre *et al*, 2004; Zeise *et al*, 1991).

Ionotropic NMDARs, cation channels with a high permeability to Ca^{2+} when activated, consist of hetero-tetrameric assemblies of NR1, NR2A-D, and NR3 subunits. The subunits are assembled cotranslationally in the endoplasmic reticulum, from where they are transported and then inserted at glutamatergic synapses. Release of NMDARs from the endoplasmic reticulum requires phosphorylation of NR1 and NR2 by protein kinases A and C (PKA and PKC), or by serine or tyrosine kinases (Scott *et al*, 2003; Wenthold *et al*, 2003). Binding of glutamate or NMDA to synaptic NMDARs allows Ca^{2+} into the cell to activate Ca^{2+}-dependent enzymes such as calcium/calmodulin-dependent protein kinase II (CaMKII), PKC, phospholipase Cγ, and phospholipase A_2 (PLA_2) (Colbran, 2004; Fukunaga *et al*, 1992; Gurd and Bissoon, 1997; Weichel *et al*, 1999; Wenthold *et al*, 2003).

Exposure of neurons from different brain regions to NMDA is reported to activate PLA_2 through the Ca^{2+} mechanism, to release the second messenger, arachidonic acid (AA, 20:4 n-6), from membrane phospholipid

*Correspondence: Dr M Basselin, Brain Physiology and Metabolism Section, National Institute on Aging, National Institutes of Health, Bldg 9, Room 1S126-MSC 0947, Bethesda, MD 20892, USA, Tel: +1 301 594 5522, Fax: +1 301 402 0074, E-mail: mirvasln@mail.nih.gov
Received 13 April 2005; revised 25 August 2005; accepted 30 August 2005
Online publication: 1 September 2005 at http://www.acnp.org/citations/Npp090105050247/default.pdf

(Dumuis *et al*, 1988; Kim *et al*, 1995; Kolko *et al*, 1999, 2003; Lazarewicz *et al*, 1990; Pellerin and Wolfe, 1991; Sanfeliu *et al*, 1990; Tapia-Arancibia *et al*, 1992; Tencé *et al*, 1994; Weichel *et al*, 1999). The AA release could be blocked by the nonspecific PLA_2 inhibitor, mepracrine, or in the rat hippocampus by bilobalide, a constituent of Ginkgo biloba, and by a specific inhibitor of $cPLA_2$ (cytosolic PLA_2) (see below) (Sanfeliu *et al*, 1990; Tapia-Arancibia *et al*, 1992; Tencé *et al*, 1994; Weichel *et al*, 1999). In contrast, NMDA did not stimulate AA release in hippocampal astroglia (Sanfeliu *et al*, 1990).

Optical methods have been used to image the Ca^{2+} signal in response to exogenous NMDA in brain slices or cell culture (Hashimoto *et al*, 2002; Ismailov *et al*, 2004; Mainen *et al*, 1999; Takita *et al*, 1997). However, no method exists, as far as we know, to image the Ca^{2+} signal or its downstream effects in the brain *in vivo*. In this paper, we present such a method, which extends our published method of imaging brain PLA_2 activation in unanesthetized rats given drugs that act at receptors coupled a G-protein to PLA_2 (Basselin *et al*, 2005a, b, 2003; Bhattacharjee *et al*, 2005; DeGeorge *et al*, 1991; Qu *et al*, 2003)—cholinergic muscarinic $M_{1,3,5}$, dopaminergic D_2-like and serotonergic $5\text{-}HT_{2A/2C}$ receptors (Axelrod, 1995; Bayon *et al*, 1997; Felder *et al*, 1990; Vial and Piomelli, 1995).

There are three classes of brain PLA_2 enzymes: AA-selective Ca^{2+}-dependent $cPLA_2$, $sPLA_2$ (secretory PLA_2), and $iPLA_2$ (Ca^{2+}-independent PLA_2) (thought to be selective for docosahexaenoic acid (DHA, 22 : 6 n-3) rather than AA). All have been identified in neurons (Kishimoto *et al*, 1999; Strokin *et al*, 2004; Yang *et al*, 1999; Yegin *et al*, 2002). $cPLA_2$ has been localized at postsynaptic neuronal membranes in the brain (Basavarajappa *et al*, 1998; Ong *et al*, 1999; Pardue *et al*, 2003) and is activated by 300 nM to 1 μM Ca^{2+} (Clark *et al*, 1995), in the physiological range of intracellular Ca^{2+} during neuronal activation (Ismailov *et al*, 2004). $sPLA_2$ is activated at much higher Ca^{2+} concentrations, 16–18 mM (Dennis, 1994), and is localized in presynaptic vesicles that are released by exocytosis during membrane depolarization (Matsuzawa *et al*, 1996; Wei *et al*, 2003).

In elaborating our *in vivo* fatty acid imaging method when stimulating receptors coupled to PLA_2 (see above), we concluded that a fraction of the AA that is released following PLA_2 activation is rapidly reincorporated into phospholipid, whereas the remainder is lost by conversion to eicosanoids or other products, or by β-oxidation (Rapoport, 2001, 2003; Robinson *et al*, 1992). Unesterified AA in plasma rapidly replaces the quantity lost, as AA is nutritionally essential and cannot be synthesized *de novo* in vertebrate tissue or elongated significantly from its precursor, linoleic acid (18 : 2 n-6) (DeMar *et al*, 2004b; Holman, 1986; Washizaki *et al*, 1994). Replacement is proportional to PLA_2 activation and can be imaged by injecting radiolabeled AA intravenously, then measuring regional brain radioactivity by quantitative autoradiography. A regional AA incorporation coefficient k^* (regional brain radioactivity/integrated plasma radioactivity), calculated in this way, has been shown to be independent of changes in cerebral blood flow and to represent the plasma-derived AA reincorporated into brain phospholipid (Basselin *et al*, 2003; Chang *et al*, 1997; DeGeorge *et al*, 1991; DeMar *et al*, 2004a; Rapoport, 2001, 2003; Robinson *et al*, 1992).

We thought it important to see if we could image brain AA signaling in response to NMDA, and if chronic LiCl would attenuate this signaling. In this study, we injected radiolabeled AA intravenously into unanesthetized rats to determine k^* for AA in each of 83 brain regions in response to i.p. saline, and to each of two i.p. doses of NMDA, 25 and 50 mg/kg, neither of which is reported to produce convulsions although capable of inducing paroxysmal EEG activity (Ormandy *et al*, 1991). We also imaged regional k^* responses to the selective noncompetitive NMDA antagonist, MK-801 ((5R,10S)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate) (Wong *et al*, 1986, 1988), given at a dose of 0.3 mg/kg i.p. alone or prior to NMDA administration. Alone, this dose is reported to produce ataxia, hyperlocomotion, and stereotypies (Dai *et al*, 1995) accompanied by cortical spike-wave discharges (Feinberg *et al*, 1995). Finally, we measured k^* for AA in response to NMDA in animals fed a control or LiCl diet for 6 weeks. The LiCl diet produces plasma and brain lithium concentrations of about 0.8 mM, reported to be therapeutic in BD (Basselin *et al*, 2003; Bosetti *et al*, 2002b; Calabrese *et al*, 1995; Chang *et al*, 1996). An abstract of part of this work has been presented (Basselin *et al*, 2004).

MATERIALS AND METHODS

Animals and Diets

Experiments were conducted following the 'Guide for the Care and Use of Laboratory Animals' (National Institute of Health Publication No. 86-23) and were approved by the Animal Care and Use Committee of the National Institute of Child Health and Development. Male Fischer CDF (F-344)/CrlBR rats (2 months old) (Charles River Laboratories, Wilmington, MA) were fed a control or LiCl diet as previously described (Basselin *et al*, 2003).

Drugs

NMDA (25 or 50 mg/kg, RBI Signaling Innovation, Sigma-Aldrich, Natick, MA), (+)-MK-801 hydrogen maleate (0.3 mg/kg, RBI Signaling Innovation) or 0.9% NaCl (saline) was administered i.p. [1-^{14}C]AA in ethanol (53 mCi/mmol, >98% pure, Moravek Biochemicals, Brea, CA) was evaporated and resuspended in HEPES buffer, pH 7.4, containing 50 mg/ml BSA, as described (DeGeorge *et al*, 1989). To confirm tracer purity, gas chromatography was performed after the [1-^{14}C]AA was converted to a methyl ester using 1 ml of 1% H_2SO_4 in anhydrous methanol (Makrides *et al*, 1994).

Surgical Procedures and Tracer Infusion

Surgery was performed as described (Basselin *et al*, 2003; Chang *et al*, 1996). A rat was wrapped loosely in a fast-setting plaster cast taped to a wooden block, and allowed to recover from anesthesia for 3–4 h. Body temperature was maintained at 36.5°C using a feedback-heating device. Arterial blood pressure and heart rate were measured

8 and 28 min after NMDA and MK-801 injection, respectively. Behavior was monitored immediately after drug administration until the end of the experiment. Repeated cycles consisting of an activity followed by a calm period were produced by NMDA. We measured the mean duration of each of these periods, as well as of the net cycling period.

At 10 min after NMDA or 30 min after MK-801 injection [1-^{14}C]AA (170 μCi/kg, 2 ml, 400 μl/min) was infused for 5 min via the femoral catheter using an infusion pump (Harvard Apparatus Model 22, Natick, MA). For studies with both drugs, MK-801 was administered 30 min prior to NMDA, which in turn was given 10 min before [1-^{14}C]AA. Timed arterial blood samples were collected during radiotracer infusion, to time of death at 20 min, when the rat was killed using an overdose of sodium pentobarbital (100 mg/kg i.v.) and immediately decapitated. The brain was removed and quickly frozen in 2-methylbutane at -40°C, stored at -80°C, and later sectioned for autoradiography.

Chemical Analysis

Plasma was separated from arterial blood by centrifugation, and lipids were extracted using a modification of Folch's method (DeGeorge *et al*, 1991; Folch *et al*, 1957). Total radioactivity in the organic phase was measured by liquid scintillation counting.

Quantitative Autoradiography

Quantitative autoradiography was performed as described previously (Basselin *et al*, 2003). A total of 83 brain regions were identified by comparing autoradiographs of coronal brain sections with a coronal atlas of the rat brain (Paxinos and Watson, 1987). The average of three bilateral optical density readings for each brain region from each animal was used to calculate regional radioactivity. Regional brain incorporation coefficients of AA from plasma into the stable brain lipid compartment, k^* (ml/s/g brain), were calculated as

$$k* = \frac{c^*_{\text{brain}}(20\,\text{min})}{\int_0^{20} c^*_{\text{plasma}}\,\mathrm{d}t} \quad (1)$$

where c^*_{brain} (20 min) nCi/g equals brain radioactivity at the time of death (20 min), c^*_{plasma} nCi/ml equals plasma AA radioactivity determined by scintillation counting, and t equals time after onset of infusion.

Statistical Analyses

In each of 83 brain regions, effects on k^* of NMDA and MK-801 alone and in combination in control diet rats were compared to effects of saline by one-way ANOVA with Dunnett's post-test with correction for four comparisons, using GraphPad Prism version 3.0a for Macintosh (GraphPad Software, San Diego CA, www.graphpad.com). Corrections for multiple comparisons across regions were not made because this was an exploratory study (Bland, 2000) to identify the regions that were involved in individual drug effects. Additionally, a two-way ANOVA, comparing Diet (LiCl *vs* control) with Drug (NMDA *vs* saline), was performed for each region using SPSS 10.0 for Macintosh (SPSS Inc., Chicago, IL, http://www.spss.com). At regions in which Diet × Drug interactions were statistically insignificant, probabilities of Main effects of Diet and Drug were separately calculated. At regions in which interactions were statistically significant, these probabilities were not calculated because they cannot be interpreted with certainty (Tabachnick and Fidell, 2001). Instead, unpaired *t*-tests were used to test for individual significant differences between means. Data are reported as means ± SD, with statistical significance taken as $p \leqslant 0.05$.

RESULTS

Physiological Parameters

At the time of surgery, the LiCl-fed animals weighed significantly less than the control diet group (257 ± 42 *vs* 299 ± 15 g, $n = 36$, $p < 0.0001$), as reported previously (Basselin *et al*, 2003).

In control diet rats, 25 mg/kg NMDA produced repeated cycles of head weaving and body movements lasting 4 ± 1 s (activity period), following by a 'calm period' lasting 9 ± 1 s (Table 1). The net time during which cycling continued was 90 ± 15 s. NMDA at 50 mg/kg also caused cycling, but with a significantly shorter 'calm period' (5 ± 2 s) than the lower dose, and a longer 'net cycling period' (180 ± 10 s). No significant difference in cycling periods was observed in animals fed the LiCl *vs* those fed the control diet. MK-801 provoked head-weaving and body movements from 5 min after injection to the end of the experiment.

NMDA at both doses significantly decreased heart rate by about 20% in both control and LiCl diet groups, but did not significantly change arterial blood pressure, whereas MK-801 increased arterial blood pressure by 30% in the control diet group (Table 1). Such changes, reported previously, have been ascribed to a centrally mediated increase in sympathetic nerve activity by MK-801 (Lewis *et al*, 1989). MK-801 given before NMDA abolished NMDA's significant effect on heart rate.

Regional Brain AA Incorporation Coefficients, k^*, in Control Diet Rats

Plasma input function. As shown by equation (1), values of k^* for individual brain regions were calculated by dividing brain radioactivity by the brain exposure to plasma [1-^{14}C]AA, the plasma input function equal to mean integrated arterial plasma radioactivity between 0 and 20 min. This function did not differ significantly between any groups. Mean integrated radioactivity, nCi/s/ml, for control diet rats: saline (159003 ± 23885), NMDA 25 mg/kg (162630 ± 23409), NMDA 50 mg/kg (155796 ± 12953), MK-801 0.3 mg/kg (167647 ± 16279), and MK-801 + NMDA 50 mg/kg (179785 ± 10361), and for the LiCl diet rats: saline (177699 ± 14678), NMDA 25 mg/kg (166619 ± 19830), NMDA 50 mg/kg (160236 ± 15150). We have reported that chronic LiCl administration does not alter plasma unesterified fatty acid concentrations, including the AA concentration (Chang *et al*, 1999).

NMDA administration to control diet-fed rats. As illustrated in Figure 1, autoradiographs of coronal brain

Table 1 Physiological Parameters and Behavior Activity Following Drug Administration in Unanesthetized Rats

	Control diet			LiCl diet		
	NMDA 25	**NMDA 50**	**MK-801**	**NMDA 50+MK-801**	**NMDA 25**	**NMDA 50**
Heart rate (beats/min)						
Before	447±32	467±26	480±0	473±21	427±20	427±20
After	360±0***	360±0***	480±0	473±21	360±0***	367±20***
Arterial blood pressure (mmHg)						
Before						
Systolic	112±9	106±5	129±10	121±4	108±5	113±7
Diastolic	83±7	83±5	84±10	71±8	83±5	82±7
After						
Systolic	109±10	101±8	167±17***	154±15***	106±8	115±7
Diastolic	80±5	78±5	113±7***	98±5***	79±3	83±7
Behavior: duration (s)						
Cycle						
Activity	4±1	4±1	ND	ND	4±1	4±1
Calm	9±1	5±2***	ND	ND	10±1	6±2***
Net cycling	90±15	180±10***	ND	ND	110±10	190±15***

ND, not determined.
Data are presented as mean±SD ($n=9$ except for MK-801+NMDA, $n=8$).
***$p<0.0001$.

sections from control rats showed that NMDA 50 mg/kg compared with saline generally increased k^* for AA whereas MK-801 generally decreased k^*. There was no apparent difference between the autoradiographs from the LiCl-fed and control diet rat at baseline (i.p. saline), whereas NMDA in the LiCl-fed rat did not appear to have an effect on k^*. Net regional data derived from the autoradiographs are presented in Tables 2 and 3 below.

As shown in Table 2, NMDA (25 mg/kg) compared with saline significantly increased k^* for AA in 49/83 regions examined (25 regions at $p<0.05$ and 24 regions at $p<0.001$). k^* was elevated significantly in the prefrontal, frontal, motor, somatosensory, auditory and visual cortical areas (18–38%), olfactory tubercle (22%), hippocampus (20–29%), caudate-putamen (17–20%), lateral geniculate nucleus dorsal (22%), regions of the thalamus (15–20%), and substantia nigra (20%), with mean increments of 21.8±5.1% in these areas. Also, 67 of 83 regions, including the nucleus accumbens (39%) (10 regions at $p<0.05$ and 57 regions at $p<0.001$), showed significant increments in k^* following 50 mg/kg NMDA compared with saline. Consistent with a dose–response relation, the mean of the significant increments equaled 30.6±8.0%, which was significantly greater ($p<0.0001$) than that following 25 mg/kg NMDA.

MK-801 in control diet rats. Compared with saline, MK-801 given to control diet rats decreased k^* for AA significantly in 21/83 regions (Table 2, Figure 1), including the frontal cortex (16%), pyriform cortex (20%), preoptic area (21%), suprachiasmatic nucleus (24%), globus pallidus (25%), hippocampus (21%), thalamus (22–26%), and hypothalamus (32–49%). The mean reduction in the significantly affected regions was 29.8±10.9%.

MK-801 followed by NMDA in control diet rats. In control diet rats, MK-801 followed by NMDA 50 mg/kg, when compared with saline decreased k^* significantly in 21/83 regions, of which 17 showed a significant decrease following MK-801 alone (see above).

Insignificant Diet × Drug interactions: As illustrated in Table 3, 18/83 regions had a statistically insignificant Diet (LiCl diet *vs* control diet) × Drug (NMDA *vs* saline) interaction with regard to k^* for AA*. In seven of the 18, NMDA compared with saline had a significant positive main Drug effect on k^*, elevating k^* to the same extent in both the control diet and LiCl-fed rats. In two of the 18 regions (preoptic area and thalamus posterior), LiCl compared with control diet had a significant main effect on k^*, elevating k^* to the same extent following saline or NMDA injection. Of the 83 regions, 10 did not have a significant Drug or Diet main effect.

Significant Diet × Drug interactions: Diet × Drug interactions were statistically significant in 65/83 regions, including the prefrontal, frontal, motor, pyriform, anterior cingulate, somatosensory and visual cortex, preoptic area, superchiasmatic nucleus, globus pallidus, hippocampus, caudate-putamen, habenular nucleus, lateral geniculate

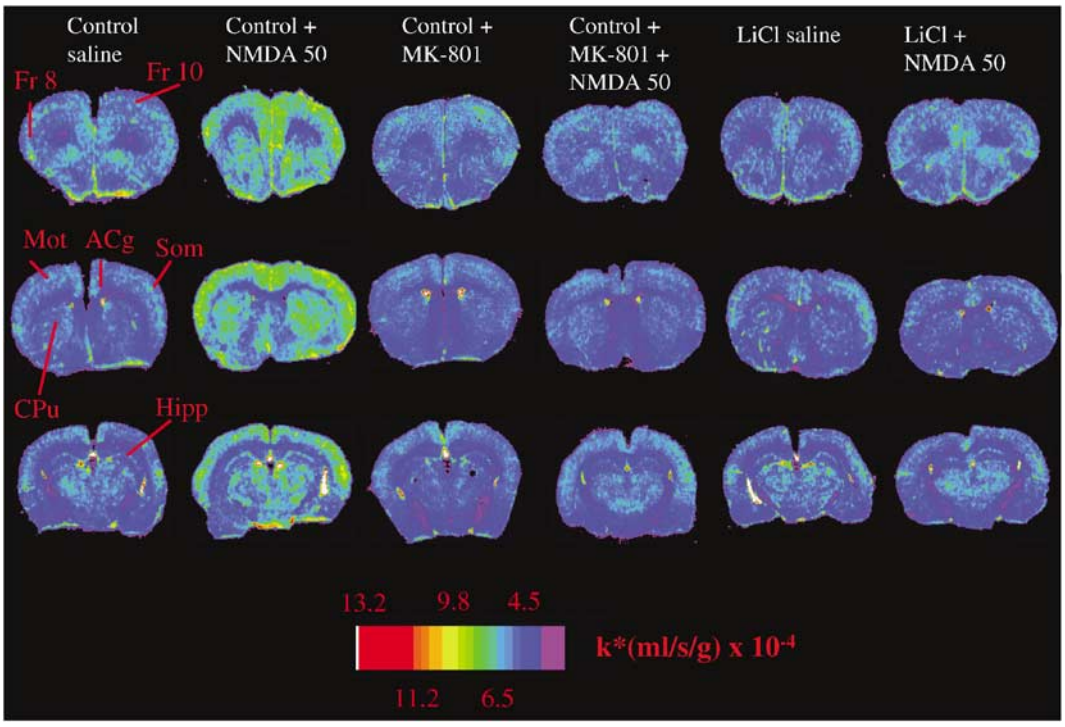

Figure 1 Effect of NMDA and MK-801 on AA incorporation into brain of control and LiCl fed rats. Regional incorporation coefficients k^* are illustrated in autoradiographs from coronal brain sections obtained 20 min after infusing [1-^{14}C]AA. Fr, frontal cortex; Mot, motor cortex; ACg, anterior cingulated cortex; Som, somatosensory cortex; CPu, caudate putamen; Hipp, hippocampus.

nucleus dorsal, geniculate medial, areas of the thalamus and hypothalamus, substantia nigra, and inferior colliculus (Table 3). In 10 of the 65 regions, unpaired *t*-tests showed that the LiCl diet, compared to the control diet, elevated k^* significantly at baseline (in response to saline). These regions included visual cortex layers I, IV, and VI, lateral habenular nucleus, medial geniculate nucleus, and inferior colliculus, which belong to central auditory and visual pathways (Brodal, 1981). We reported this observation (Basselin *et al*, 2003, 2005b), and suggested that it may underlie lithium's ability in human subjects to increase the amplitudes of P1/N1 components of auditory-evoked responses and of 65-P95 and P95-N125 components of visual-evoked responses (Fenwick and Robertson, 1983; Hegerl *et al*, 1990; Ulrich *et al*, 1990).

In 65/83 regions, NMDA increased k^* significantly in the control diet but not in the LiCl-fed rats. Thus, LiCl blocked 97% of the positive k^* responses to 50 mg/kg NMDA (65/67).

DISCUSSION

This study shows that (1) the *in vivo* fatty acid method (see Introduction) (Rapoport, 2001, 2003) can be used to image PLA_2 activation coupled to NMDARs in unanesthetized rats; (2) regional increments in k^* for AA in response to subconvulsant NMDA doses in control diet rats are blocked by pretreatment with MK-801; (3) MK-801 alone (or when given prior to 50 mg/kg NMDA) produces widespread baseline reductions in k^* for AA in control diet rats; and (4) a 6-week LiCl diet, sufficient to produce plasma and brain lithium concentrations therapeutically relevant to BD (Bosetti *et al*, 2002b; Chang *et al*, 1996), largely (97%) prevents the significant NMDA-induced increments in k^* seen in control diet rats.

NMDA at 25 and 50 mg/kg significantly increased k^* for AA by an average of 22% and 31%, respectively, in significantly affected brain regions. These regions, reported to have NMDARs (Bernard and Bolam, 1998; Ikeda *et al*, 2003; Jarvis *et al*, 1987; Monaghan and Cotman, 1986; Pal *et al*, 1999; Petralia *et al*, 1994), included cerebral cortex, caudate-putamen, globus pallidus, hippocampus, substantia nigra, and nucleus accumbens. NMDA has also been reported to increase the regional cerebral metabolic rate for glucose, $rCMR_{glc}$, a marker of axonal activity (Sokoloff, 1999), in the rat suprachiasmatic nucleus (Shibata *et al*, 1992), frontal cortex layers V-VI, globus pallidus, caudate-putamen, substantia nigra, thalamus, nucleus accumbens, and lateral habenular nucleus (Browne *et al*, 1998).

The blocking of increments in k^* for AA following NMDA by MK-801 is consistent with the increments being mediated by NMDARs. Furthermore, the ability of MK-801, given alone or prior to NMDA, to significantly reduce k^* for AA by 16–49%, suggests that NMDA-initiated PLA_2 activation contributes substantially to the baseline release of AA in control brain. This interpretation is consistent with evidence that glutamatergic receptors constitute about 75% of the synapses in the mammalian cortex, and that many of these synapses include NMDARs (Braitenberg and Schüz, 1998; Fonnum, 1984; Raichle and Gusnard, 2002).

The increments in k^* for AA in response to the subconvulsant doses of NMDA were most likely due to enhanced Ca^{2+} entry into the cell via NMDARs, activating

Table 2 Mean Values of Arachidonic Acid Incorporation Coefficients k^*[a] in Control Diet-Fed Rats, at Baseline (in Response to Saline) and in Response to NMDA[b], MK-801[c], and MK-801+NMDA[d]

	Control diet				
Brain region	**Saline ($n=9$)**	**NMDA 25 ($n=9$)**	**NMDA 50 ($n=9$)**	**MK-801 ($n=9$)**	**MK-801+NMDA 50 ($n=8$)**
Telencephalon					
Prefrontal cortex layer I	6.15±0.54	7.64±0.79***	7.81±0.83***	5.58±0.36*	5.77±0.58
Prefrontal cortex layer IV	6.41±0.62	8.11±0.56***	8.22±0.94***	6.35±0.44	6.57±0.58
Primary olfactory cortex	5.90±0.82	7.42±0.93***	8.04±0.94***	4.98±0.47	4.72±0.69*
Frontal cortex (10)					
Layer I	6.37±0.61	7.76±0.64***	8.62±1.06***	5.69±0.40	5.75±0.59
Layer IV	6.77±0.68	8.18±0.64***	8.87±0.90***	6.85±0.63	6.99±0.43
Frontal cortex (8)					
Layer I	6.46±0.59	8.04±0.69***	8.43±1.00***	5.42±0.48***	5.39±0.41***
Layer IV	6.89±0.68	8.58±0.79***	8.95±1.13***	6.06±0.51	5.98±0.31
Pyriform cortex	5.64±0.52	6.29±0.73	6.93±0.82***	4.53±0.61***	4.13±0.77***
Anterior cingulate cortex	7.34±1.10	8.59±0.64*	9.87±1.29***	6.60±0.17	7.01±1.22
Motor cortex					
Layer I	6.43±0.67	7.57±0.54*	8.35±1.35***	5.95±0.08	5.64±0.90
Layer II–III	6.54±0.68	7.78±0.49*	8.34±1.33***	6.03±0.33	6.05±0.86
Layer IV	6.88±0.77	8.40±0.81***	9.22±1.36***	6.60±0.38	6.46±0.92
Layer V	5.31±0.44	6.54±0.37***	7.14±1.00***	4.99±0.33	4.86±0.61
Layer VI	5.25±0.55	6.24±0.36***	6.90±0.93***	4.73±0.32	4.86±0.68
Somatosensory cortex					
Layer I	6.32±0.66	7.83±0.54*	9.15±1.6***	5.61±0.26	5.38±0.92
Layer II–III	6.36±0.75	7.86±0.56***	9.00±1.30***	5.74±0.28	5.81±0.77
Layer IV	6.93±0.59	8.65±0.80*	9.79±1.84***	6.25±0.31	6.30±0.94
Layer V	6.22±0.73	7.72±0.53*	8.99±1.62***	5.70±0.42	5.74±0.80
Layer VI	6.16±0.85	7.32±0.44*	8.29±1.53***	5.60±0.51	5.70±0.90
Auditory cortex					
Layer I	6.37±0.39	7.53±1.08	7.75±2.37	6.29±0.36	5.63±0.23
Layer IV	7.06±0.41	8.45±1.05	8.74±2.31*	6.74±1.14	6.17±0.64
Layer VI	6.02±0.59	7.95±0.89***	8.01±1.55***	6.99±0.71	6.69±0.41
Visual cortex					
Layer I	5.28±0.92	7.29±1.00***	7.50±1.35***	6.12±0.95	5.83±0.94
Layer IV	6.07±0.54	7.74±0.94***	7.97±1.54***	6.51±0.81	6.31±0.96
Layer VI	5.64±0.55	7.09±0.86***	7.36±1.24***	5.93±0.98	5.86±1.16$^{\Delta}$
Preoptic area (LPO/MPO)	5.70±0.88	6.56±0.71	6.82±0.69*	4.49±0.66***	4.46±0.88***
Suprachiasmatic nu	5.53±0.77	6.50±0.77*	5.55±0.99	4.23±0.61***	3.92±0.65***
Globus pallidus	5.31±0.80	6.38±0.75*	6.42±0.69***	3.98±0.73***	3.92±0.67***
Bed nu stria terminalis	5.68±0.82	6.44±0.94	6.98±0.50***	4.16±0.56***	4.12±0.49***
Olfactory tubercle	5.83±0.57	7.12±0.55***	7.42±0.93***	5.13±0.39	5.04±0.69
Diagonal band					
Dorsal	5.75±0.33	7.04±0.82***	8.04±0.57***	4.94±0.70	5.10±0.76
Ventral	5.61±0.60	6.96±0.84***	8.04±0.87***	4.99±0.67	4.70±0.64
Amygdala basolat/med	5.28±0.95	5.88±0.51	6.42±0.64***	4.23±0.77*	4.18±0.72*

Table 2 Continued

Brain region	Control diet				
	Saline ($n=9$)	NMDA 25 ($n=9$)	NMDA 50 ($n=9$)	MK-801 ($n=9$)	MK-801+NMDA 50 ($n=8$)
Hippocampus					
CA1	4.70±0.53	5.95±0.70***	6.16±0.44***	4.13±0.43	4.30±0.49
CA2	4.99±0.78	6.03±0.61***	6.58±0.44***	3.93±0.51***	4.13±0.53*
CA3	5.34±0.93	6.23±0.68*	6.66±0.44***	4.20±0.56***	4.48±0.56*
Dentate gyrus	5.74±0.83	6.52±0.64	7.54±1.16***	5.28±0.98	5.69±0.81
SLM	5.48±0.75	7.08±0.84***	8.08±1.19***	5.43±0.46	5.94±0.47
Accumbens nucleus	5.92±0.78	6.19±0.56	8.24±1.57***	5.62±0.66	5.92±0.50
Caudate putamen					
Dorsal	6.17±0.57	7.21±0.65***	8.22±0.86***	5.71±0.47	5.79±0.49
Ventral	6.11±0.64	7.12±0.66*	8.25±1.04***	5.57±0.65	5.87±0.55
Lateral	6.06±0.60	7.28±0.68*	8.32±1.15***	5.72±0.63	6.22±0.76
Medial	6.12±0.69	7.26±0.70*	8.21±0.96***	5.51±0.71	5.78±0.67
Septal nucleus lateral	5.61±0.74	6.16±0.63	7.28±1.00***	4.34±0.99***	4.20±0.82***
Medial	5.93±0.49	6.29±0.71	7.88±0.92***	4.90±0.90*	5.01±0.70*
Diencephalon					
Habenular nu lateral	7.00±0.95	8.33±1.43	9.50±2.63*	6.52±1.00	7.12±0.43
Medial	7.22±0.78	8.01±1.23	9.45±2.65*	6.53±0.99	6.19±0.82
Lat geniculate nu dorsal	6.42±0.46	7.85±0.99***	8.92±1.03***	6.36±0.70	6.17±0.61
Geneculate medial	6.28±0.81	7.74±0.94	7.96±2.08*	6.04±0.52	6.21±0.71
Thalamus					
Ventroposterior lat nu	6.28±0.47	6.79±0.75	7.52±0.79***	5.52±0.93	5.90±0.86
Ventroposterior med nu	6.09±0.44	6.72±0.69	7.54±0.64***	5.60±0.94	5.44±0.55
Paratenial nu	6.52±0.67	7.48±0.71	8.64±1.31***	4.88±0.52***	5.37±0.73*
Anteroventral nu	7.68±1.10	9.25±1.04*	10.74±1.75***	6.18±0.69	7.19±1.00
Anteromedial nu	6.51±0.66	7.80±0.71***	8.66±0.93***	5.06±0.58***	5.67±0.55
Reticular nu	6.55±0.56	7.77±0.81*	8.95±0.91***	5.14±0.61***	6.13±1.17
Paraventricular nu	6.58±0.72	7.65±0.67*	8.64±1.28***	4.84±0.50***	5.35±0.54*
Parafascicular nu	6.06±0.56	6.94±0.63*	7.03±0.64***	6.10±1.03	5.93±0.55
Subthalamic nucleus	6.51±0.61	7.52±1.07*	8.33±0.82***	6.90±0.93	6.70±0.28
Hypothalamus					
Supraoptic nu	5.31±0.60	6.28±0.75*	6.76±0.99***	4.16±1.11	5.28±0.60
Lateral	5.93±0.61	6.59±0.72	6.72±0.84*	4.05±0.58***	4.24±0.21***
Anterior	5.79±0.63	6.56±0.83	6.72±0.82*	3.94±0.64***	4.37±0.17***
Periventricular	6.34±0.39	6.87±0.81	7.84±0.97***	3.21±0.55***	3.58±0.20***
Arcuate	5.79±1.12	5.88±0.75	6.59±0.70	3.36±1.01***	3.10±1.32***
Ventromedial	5.61±0.96	6.01±0.63	6.50±0.55*	3.49±0.93***	3.16±1.16***
Posterior	6.34±0.39	6.87±0.81	7.84±0.97***	5.03±1.28	3.37±1.12***
Mammillary nucleus	6.03±0.78	6.82±0.90	7.10±1.30	5.64±0.92	6.83±0.94
Mesencephalon					
Interpeduncular nucleus	8.18±0.69	9.62±1.32	9.70±2.33	7.89±0.24	7.51±0.61
Substantia nigra					
Pars reticulata	5.57±0.43	6.57±0.46*	6.39±0.60	5.16±1.47	4.66±0.47
Pars compacta	5.57±0.47	6.74±0.56*	6.42±0.57	5.11±1.47	4.53±0.42*
Pretectal area	6.39±0.61	7.56±0.93	6.88±0.57	6.38±0.99	6.36±0.76

Table 2 Continued

Brain region	Control diet				
	Saline ($n=9$)	**NMDA 25 ($n=9$)**	**NMDA 50 ($n=9$)**	**MK-801 ($n=9$)**	**MK-801+NMDA 50 ($n=8$)**
Superior colliculus	5.68±0.91	6.95±0.75*	7.24±1.57*	5.42±1.16	5.60±0.50
Deep layers	6.60±0.60	7.97±0.90	7.71±2.16	6.45±1.21	6.02±0.99
Inferior colliculus	7.18±0.64	9.82±0.66*	10.57±1.38***	7.83±0.96	8.28±0.47
Rhombencephalon					
Flocculus	6.97±0.46	7.87±0.96	7.58±1.33	5.81±1.00	6.16±0.61
Cerebellar gray matter	5.96±0.88	6.88±0.66*	6.63±1.11*	5.80±0.86	5.85±0.37
Molecular layer cerebellar gray matter	7.18±0.90	8.77±1.08	8.82±2.22	7.86±1.03	8.42±0.44
White matter					
Corpus callosum	4.78±0.71	5.48±0.60*	5.91±0.40***	4.34±0.77	4.04±0.67
Zone incerta	5.48±0.55	6.04±0.62	6.38±0.38***	5.14±0.90	4.53±0.51
Internal capsule	4.41±1.02	4.99±1.31	5.21±0.47	3.40±0.71	3.47±0.51
Cerebellar white matter	3.29±1.03	3.42±0.58	4.26±0.76	2.87±0.98	3.35±0.71
Nonblood–brain barrier regions					
Subfornical organ	5.31±0.60	5.28±0.75	5.76±0.99	5.13±1.27	4.62±0.87
Median eminence	5.72±0.84	6.58±1.06	6.56±0.55	4.06±1.09	5.22±0.44
Choroid plexus	15.2±1.82	17.2±2.81	16.4±2.73	15.4±2.82	15.7±0.81

nu, nucleus; lat, lateral; med, medial; SLM, stratum lacunosum-moleculare of the hippocampus.
Each value is a mean±SD.
*$p<0.05$; ***$p<0.001$; compared with the saline group (one-way ANOVA Dunnett tests).
[a]$k^* = (\text{ml/s/g}) \times 10^{-4}$.
[b]NMDA administration: 25 and 50 mg/kg i.p., 10 min.
[c]MK-801 administration: 0.3 mg/kg i.p., 30 min.
[d]MK-801+NMDA administration: 0.3 mg/kg i.p., 30 min followed by NMDA 50 mg/kg i.p., 10 min.

$cPLA_2$ to release AA from membrane phospholipid. NMDA-provoked $EPLA_2$ activation has been demonstrated in rat hippocampal slices, where it could be blocked by a specific $cPLA_2$ inhibitor and was accompanied by the formation of *lyso*-phosphatidylcholine and glycerophosphocholine, consistent with activation of the AA-selective $cPLA_2$ (Dennis, 1994; Weichel *et al*, 1999). Other studies also reported that Ca^{2+}-mediated activation of PLA_2 in neurons released AA following NMDA (Dumuis *et al*, 1988; Lazarewicz *et al*, 1990; Pellerin and Wolfe, 1991; Tapia-Arancibia *et al*, 1992). Although both $cPLA_2$ and $sPLA_2$ were activated in some of those studies (Kim *et al*, 1995), it is likely that mainly $cPLA_2$ was activated in ours, as $cPLA_2$ is sensitive to the low Ca^{2+} concentrations, 300 nM to 1 μM (Clark *et al*, 1995; Ismailov *et al*, 2004) that occur during normal neuronal activation. $sPLA_2$ activation requires a much higher Ca^{2+} concentration, 16–18 mM, and is involved mainly in the presynaptic release of synaptic vesicles (Dennis, 1994; Matsuzawa *et al*, 1996; Wei *et al*, 2003). $iPLA_2$ is Ca^{2+}-independent and selective for DHA acid rather than AA (Dennis, 1994; Strokin *et al*, 2004).

The chronic LiCl diet of this study is reported to reduce global brain mRNA, protein and activity levels of $cPLA_2$ in rats, but not to affect expression of $sPLA_2$ or $iPLA_2$ (Bosetti *et al*, 2002a, b; Chang and Jones, 1998; Rapoport and Bosetti, 2002; Rintala *et al*, 1999; Weerasinghe *et al*, 2004). Its $cPLA_2$ effect may be due a lithium-induced downregulation of Ca^{2+}-dependent PKC, to reduce expression of PKC-dependent AP-2, which regulates $cPLA_2$ transcription (Rao *et al*, 2005; Wang *et al*, 2001). Consistent with selective $cPLA_2$ targeting, the LiCl diet reduces incorporation and turnover rates of AA but not of DHA in brain phospholipids of unanesthetized rats (Chang *et al*, 1996). Thus, one explanation for its suppression of the NMDA-induced increments in k^* for AA in the present study is that it directly reduces the $cPLA_2$ activation response to increased intracellular Ca^{2+}.

Another possible explanation is that it changed the phosphorylation state of NMDAR subunits, preventing NMDAR-mediated Ca^{2+} entry into cells. For example, exposing cultured neurons to lithium, or injecting lithium into rats for 7 days, has been reported to reduce NMDAR-mediated Ca^{2+} influx by inhibiting NR2B tyrosine phosphorylation and Src tyrosine kinase (Hashimoto *et al*, 2002, 2003; Nonaka and Chuang, 1998), or by reducing NR2A tyrosine phosphorylation and interactions of the NR2A subunit with Src and Fyn mediated by PSD-95 in the rat hippocampus (Ma and Zhang, 2003; Ma *et al*, 2004). If blocking NMDAR-mediated Ca^{2+} entry into neurons were the primary reason for the reduced k^* responses, then lithium's reduction of global $cPLA_2$ expression in the rat

Table 3 Mean Values of Arachidonic Acid Incorporation Coefficients k^*[a] in Lithium Diet-Fed Rats, at Baseline (in Response to Saline) and in Response to NMDA[b]

	LiCl diet					
Brain region	**Saline ($n=9$)**	**NMDA 25 ($n=9$)**	**NMDA 50 ($n=9$)**	**Diet × drug interaction**	**LiCl effect**	**Drug effect**
Telencephalon						
Prefrontal cortex (10)-I	5.70±0.73	5.87±0.88	5.32±0.83	<0.001		
Prefrontal cortex (10)-IV	6.30±0.83	6.20±0.91	6.03±1.03	<0.001		
Primary olfactory cortex	5.57±0.96	5.47±0.79	5.20±0.99	<0.001		
Frontal cortex (10)						
Layer I	5.94±0.80	6.09±0.87	5.58±0.91	<0.001		
Layer IV	6.47±0.81	6.54±0.96	6.06±0.95	<0.001		
Frontal cortex (8)						
Layer I	6.09±0.97	6.11±0.96	5.83±1.20	0.002		
Layer IV	6.88±1.00	6.99±0.88	6.24±0.22	<0.001		
Pyriform cortex	4.62±0.68	4.67±0.66	4.11±0.93	<0.001		
Anterior cingulate cortex	7.05±1.12	7.50±0.55	6.76±1.64	0.001		
Motor cortex						
Layer I	6.12±0.87	6.41±0.61	5.23±1.09	<0.001		
Layer II–III	6.15±0.88	6.21±0.98	5.56±1.05	0.002		
Layer IV	6.54±0.86	6.75±1.07	5.98±1.13	<0.001		
Layer V	4.92±0.81	5.12±0.71	4.69±0.98	0.001		
Layer VI	4.82±0.67	4.95±0.59	4.33±0.88	<0.001		
Somatosensory cortex						
Layer I	6.09±0.81	6.39±0.77	5.12±0.64	NS	NS	0.005
Layer II–III	6.06±0.97	6.56±0.87	5.24±0.47	NS	NS	0.002
Layer IV	6.83±1.06	6.99±0.82	5.96±1.08	NS	NS	0.015
Layer V	5.86±1.03	6.11±0.99	5.06±0.80	NS	NS	0.010
Layer VI	5.84±1.06	5.95±0.95	5.02±1.31	0.001		
Auditory cortex						
Layer I	6.36±0.84	7.53±0.94	6.95±1.60	NS	NS	0.029
Layer IV	7.20±0.55	8.78±1.10	8.10±1.52	NS	NS	0.003
Layer VI	6.29±0.63	7.27±0.91	7.00±1.17	NS	NS	NS
Visual cortex						
Layer I	6.28±0.43**	6.66±0.83	6.82±0.49	0.009		
Layer IV	7.51±0.81***	6.79±0.85	6.78±0.77	<0.001		
Layer VI	6.95±0.93	6.42±0.87	6.45±0.73	NS	NS	0.008
Preoptic area (LPO/MPO)	5.16±0.76	5.23±1.04	4.48±1.21	0.016		
Suprachiasmatic nu	5.05±0.67	5.41±1.19	4.54±1.01	NS	0.001	0.014
Globus pallidus	4.68±0.67	4.66±0.75	4.00±0.77	0.003		
Bed nu stria terminalis	4.86±0.83	4.88±0.89	4.37±1.04	0.010		
Olfactory tubercle	5.47±0.68	5.66±0.48	5.51±0.72	0.003		
Diagonal band						
Dorsal	5.52±0.89	5.62±0.91	5.10±0.95	NS	NS	0.003
Ventral	5.42±0.79	5.40±0.90	5.35±1.30	0.001		
Amygdala basolat/med	4.29±1.00	4.54±0.43	5.02±0.72	NS	NS	0.002

Table 3 Continued

Brain region	LiCl diet					
	Saline ($n=9$)	**NMDA 25 ($n=9$)**	**NMDA 50 ($n=9$)**	**Diet × drug interaction**	**LiCl effect**	**Drug effect**
Hippocampus						
CA1	4.22±0.66	4.20±0.35	4.25±0.55	<0.0001		
CA2	4.48±0.61	4.51±0.29	4.59±0.65	0.001		
CA3	4.67±0.63	4.71±0.39	4.80±0.66	0.025		
Dentate gyrus	5.22±0.89	5.14±0.66	5.42±1.02	0.033		
SLM	6.47±0.52	5.69±0.48	5.65±0.37	NS	NS	0.003
Accumbens nucleus	5.33±1.06	5.87±1.03	5.70±1.21	0.006		
Caudate putamen						
Dorsal	5.49±0.95	5.89±0.72	5.11±0.94	NS	NS	0.006
Ventral	5.57±1.00	5.87±0.74	5.28±1.00	<0.0001		
Lateral	5.48±0.88	5.84±0.79	5.15±1.13	<0.0001		
Medial	5.41±0.85	5.80±0.74	5.13±1.15	0.001		
Septal nucleus lateral	4.90±0.57	4.90±0.88	4.05±.84	NS	NS	NS
Medial	5.06±1.09	4.81±1.05	4.95±0.87	0.003		
Diencephalon						
Habenular nu lateral	8.12±0.51**	8.22±0.74	8.09±0.54	0.027		
Medial	7.92±0.44	8.30±0.72	7.93±0.60	0.032		
Lat geniculate nu dorsal	6.89±0.47	6.82±0.55	7.01±0.47	NS	NS	NS
Geniculate medial	7.25±0.66*	6.90±0.54	6.97±36	0.013		
Thalamus						
Ventroposterior lat nu	5.31±0.86	5.95±0.46	5.98±0.91	NS	NS	NS
Ventroposterior med nu	5.22±0.925	.95±0.46	6.20±0.71	NS	NS	NS
Paratenial nu	6.07±0.80	6.30±0.73	6.36±0.92	0.012		
Anteroventral nu	7.89±1.08	8.66±0.90	7.60±1.15	NS	0.001	0.002
Anteromedial nu	6.04±1.00	6.48±0.80	5.80±1.38	0.002		
Reticular nu	6.28±0.91	6.54±1.10	5.87±1.17	<0.0001		
Paraventricular nu	6.18±0.89	6.76±0.34	6.58±1.03	NS	0.0001	0.002
Parafascicular nu	5.91±0.64	5.38±0.42	5.98±0.51	0.002		
Subthalamic nucleus	5.86±0.80	5.94±0.63	6.36±1.03	NS	NS	NS
Hypothalamus						
Supraoptic nu	5.27±0.83	4.16±0.63	4.29±1.10	<0.0001		
Lateral	4.98±0.94	4.90±0.66	4.41±0.85	0.038		
Anterior	4.95±0.78	5.03±0.47	4.33±0.82	0.011		
Periventricular	4.15±0.65	4.19±0.46	3.85±0.67	NS	NS	NS
Arcuate	5.92±0.76	5.85±1.13	4.79±0.82	0.003		
Ventromedial	5.73±0.64	5.48±1.09	4.35±0.86	<0.0001		
Posterior	4.85±0.34	5.03±0.56	5.61±0.54	NS	NS	0.002
Mammillary nucleus	5.13±0.51	5.74±0.70	6.64±0.88	NS	0.001	<0.0001
Mesencephalon						
Interpeduncular nucleus	9.08±1.12	10.09±1.19	9.98±0.51	NS	NS	0.02
Substantia nigra						
Pars reticulata	5.72±0.51	5.19±0.58	5.61±0.54	<0.0001		
Pars compacta	5.65±0.75	5.39±0.44	5.48±0.60	0.004		
Pretectal area	6.88±0.71	6.06±0.67	6.45±0.70	0.001		

Table 3 Continued

Brain region	LiCl diet					
	Saline ($n=9$)	NMDA 25 ($n=9$)	NMDA 50 ($n=9$)	Diet × drug interaction	LiCl effect	Drug effect
Superior colliculus	7.23±0.52	6.41±0.68	6.40±0.88	0.001		
Deep layers	7.14±1.01	6.83±0.61	6.87±0.34	NS	NS	NS
Inferior colliculus	9.86±1.07	8.65±0.62	8.48±1.25	NS	NS	NS
Rhombencephalon						
Flocculus	8.89±2.08	8.83±1.10	9.59±0.92	NS	NS	NS
Cerebellar gray matter	6.00±0.63	5.91±0.90	6.22±0.42	0.041		
Molecular layer cerebellar gray matter	8.13±1.02	7.94±0.64	8.02±0.95	NS	NS	NS
White matter						
Corpus callosum	3.97±0.74	4.44±0.74	3.94±0.71	0.027		
Zone incerta	4.24±0.25	3.95±0.37	4.70±1.41	NS	NS	0.015
Internal capsule	3.17±0.85	3.42±0.65	3.37±0.82	NS	NS	NS
Cerebellar white matter	3.10±0.42	3.11±0.43	3.98±0.62	0.027		
Nonblood–brain barrier regions						
Subfornical organ	4.80±0.45	4.71±1.06	4.19±0.60	NS	NS	NS
Median eminence	4.95±0.72	4.73±0.34	5.00±0.74	NS	NS	NS
Choroid plexus	22.2±3.25	21.6±2.20	24.0±2.14	NS	NS	NS

nu, nucleus; lat, lateral; med, medial; SLM, stratum lacunosum-molecular of the hippocampus.
Each value is a mean±SD.
NS: not significant.
In cases of statistically significant Diet × Drug interaction, unpaired t tests were realized. *$p<0.05$; **$p<0.01$; ***$p<0.001$, LiCl diet-saline *vs* control diet-saline. No mean k^* in response to 25 or 50 mg/kg NMDA in the LiCl-fed rats differed significantly from k^* in response to saline.
[a]$k^* = (\text{ml/s/g}) \times 10^{-4}$.
[b]NMDA administration: 25 and 50 mg/kg i.p., 10 min.

brain (see above) could be secondary to a chronic down-regulation of Ca^{2+} entry via defective NMDARs (Du *et al*, 2004; Gray *et al*, 2003; Karkanias and Papke, 1999). In any case, blocking NMDAR signaling via $cPLA_2$ as contributing to lithium's therapeutic efficacy is consistent with evidence that other anti-BD drugs (Calabrese *et al*, 1995)—lamotrigine (Farber *et al*, 2002; McIntyre *et al*, 2004), carbamazepine (Hough *et al*, 1996; Kubota *et al*, 1994), and valproic acid (Zeise *et al*, 1991) have direct or indirect NMDAR-blocking properties.

Reduced NMDAR signaling as contributing to lithium's efficacy is consistent with evidence of disturbed NMDAR subunit expression in the BD brain. NR1 and NR2 genes are reported to confer susceptibility to BD (Itokawa *et al*, 2003; Mundo *et al*, 2003), and NMDAR density is decreased in the post-mortem BD hippocampus (Scarr *et al*, 2003). In other studies, NR2D mRNA was higher in the post-mortem BD striatum (Meador-Woodruff *et al*, 2001), NR1 mRNA was decreased in the BD dentate gyrus and CA3 of the hippocampus (Law and Deakin, 2001), and NR3A mRNA was decreased in the BD prefrontal cortex (Mueller and Meador-Woodruff, 2004).

It remains possible that the NMDA response involved neuroreceptors other than NMDA. For example, dopaminergic and glutamatergic/NMDA transmission are closely linked in the mammalian brain, particularly in the prefrontal cortex, striatum, and nucleus accumbens (Ferretti *et al*, 2005; Ikeda *et al*, 2003; Piomelli and Di Marzo, 1993; Rogue *et al*, 1990; Takeuchi *et al*, 2002; Tseng and O'Donnell, 2004; Wang *et al*, 2003). Medium-sized spiny neurons, the majority of neurons in the neostriatum, receive convergent glutamate-containing afferents from the neocortex and dopamine-containing afferents from the substantia nigra and ventral tegmentum (Murata *et al*, 2002). Dopamine receptor-linked signaling can affect the phosphorylation state and synaptic efficacy of NMDARs coexpressed on striatal neurons (Cepeda and Levine, 1998; Chase, 2004; Nair *et al*, 1998), whereas inhibition of NMDAR expression by antisense oligonucleotides can modulate pre- and postsynaptic expression of D_2-like receptors in these neurons (Murata *et al*, 2002). Furthermore, both dopaminergic and glutamatergic pathways can modulate neuroplasticity involving cAMP, DARPP-32 (dopamine, cAMP-regulated phosphoprotein of 32 kDa), and ERK (extracellular signal-regulated kinase) (Gould and Manji, 2005; Valjent *et al*, 2005). In this regard, chronic lithium increased the level of DARPP-32 in the rat frontal cortex (Guitart and Nestler, 1992), which may explain its ability to activate the ERK-signaling cascade (Einat *et al*, 2003).

In view of the interplay between D_2-like receptors and NMDARs, it is not surprising that chronic LiCl suppresses increments in k^* for AA in unanesthetized rats given quinpirole (Basselin *et al*, 2005a), an agonist of D_2-like receptors coupled to PLA_2 via a G-protein (Bhattacharjee *et al*, 2005; Hayakawa *et al*, 2001; Huo and Healy, 1991; Vial and Piomelli, 1995). This suppression agrees with evidence that drugs that interfere with dopaminergic neurotransmission can be effective in BD (Bunney and Garland-Bunney, 1987; Bymaster and Felder, 2002; Fisher *et al*, 1991; Peet and Peters, 1995; Post *et al*, 1980; Sultzer and Cummings, 1989). On the other hand, the ability of chronic LiCl to potentiate k^* responses in rats to the muscarinic $M_{1,3,5}$ receptor agonist, arecoline (Basselin *et al*, 2003), is consistent with reports that cholinomimetics can be therapeutic as well (Bunney and Garland-Bunney, 1987; Bymaster and Felder, 2002). These published data, and the data of this study, suggest that lithium acts in BD by rectifying several neurotransmitter-signaling imbalances through downregulating NMDAR and D_2-like receptor signaling and upregulating $M_{1,3,5}$ receptor signaling involving PLA_2 and the AA cascade (Bunney and Garland-Bunney, 1987; Bymaster and Felder, 2002; Dumuis *et al*, 1988; Rapoport and Bosetti, 2002; Shimizu and Wolfe, 1990).

Glutamate at excessive concentrations can kill neurons by the process of excitotoxicity (Coyle and Puttfarcken, 1993; Rothman and Olney, 1995), and it has been suggested that glutamatergic excitotoxicity contributes to BD (Zarate *et al*, 2003). Lithium's ability to block NMDA-initiated increments in k^* for AA (this paper) is consistent with evidence that chronic lithium can be neuroprotective in animal models of neurodegeneration and even in BD patients (Moore *et al*, 2000; Rowe and Chuang, 2004; Zarate *et al*, 2003). Excess PLA_2-mediated release of AA, with concurrent formation of excess *lyso*-phospholipids, can engender neurotoxic sequellae (Bazan *et al*, 1995; Farooqui *et al*, 1997; Sapirstein and Bonventre, 2000).

Finally, interference in the normal NMDAR-initiated PLA_2-mediated release of AA by lithium may disturb long-term potentiation in the hippocampus, considered the basis of learning and memory (Dumuis *et al*, 1988; Nishizaki *et al*, 1999; Otmakhov *et al*, 2004; Schmitt *et al*, 2005; Williams *et al*, 1989). This may be relevant to reports that lithium treatment inhibited learning, memory, and speed of information processing in BD patients and to some extent in control subjects (Honig *et al*, 1999; Pachet and Wisniewski, 2003; Stip *et al*, 2000).

REFERENCES

Axelrod J (1995). Phospholipase A_2 and G proteins. *Trends Neurosci* **18**: 64–65.

Barchas J, Hamblin M, Malenka R (1994). Biochemical hypotheses of mood and anxiety disorders. In: Siegel GJ, Agranoff BW, Albers RW, Molinoff PB (eds). *Basic Neurochemistry*, 5th edn. Raven Press: New York. pp 979–1001.

Basavarajappa BS, Cooper TB, Hungund BL (1998). Effect of chronic ethanol exposure on mouse brain arachidonic acid specific phospholipase A_2. *Biochem Pharmacol* **55**: 515–521.

Basselin M, Chang L, Bell J, Rapoport SI (2005a). Chronic lithium chloride administration to unanesthetized rats attenuates brain dopamine D2-like receptor initiated signaling via arachidonic acid. *Neuropsychopharmacology* **30**: 1064–1075.

Basselin M, Chang L, Seemann R, Bell JM, Rapoport SI (2003). Chronic lithium administration potentiates brain arachidonic acid signaling at rest and during cholinergic activation in awake rats. *J Neurochem* **85**: 1553–1562.

Basselin M, Chang L, Seemann R, Bell JM, Rapoport SI (2004). Lithium treatment of rats may reduce brain NMDA-initiated arachidonic acid signaling by downregulating Ca^{2+}-dependent cytosolic phospholipase A_2. *Neuropsychopharmacology* **29** (Suppl 1): S181.

Basselin M, Chang L, Seemann R, Bell JM, Rapoport SI (2005b). Chronic lithium administration to rats selectively modifies 5-$HT_{2A/2C}$ receptor-mediated brain signaling via arachidonic acid. *Neuropsychopharmacology* **30**: 461–472.

Bauer M, Alda M, Priller J, Young LT (2003). Implications of the neuroprotective effects of lithium for the treatment of bipolar and neurodegenerative disorders. *Pharmacopsychiatry* **36**(Suppl 3): S250–S254.

Bayon Y, Hernandez M, Alonso A, Nunez L, Garcia-Sancho J, Leslie C *et al* (1997). Cytosolic phospholipase A_2 is coupled to muscarinic receptors in the human astrocytoma cell line 1321N1: characterization of the transducing mechanism. *Biochem J* **323**: 281–287.

Bazan NG, Rodriguez de Turco EB, Allan G (1995). Mediators of injury in neurotrauma: intracellular signal transduction and gene expression. *J Neurotrauma* **12**: 791–814.

Bernard V, Bolam JP (1998). Subcellular and subsynaptic distribution of the NR1 subunit of the NMDA receptor in the neostriatum and globus pallidus of the rat: co-localization at synapses with the GluR2/3 subunit of the AMPA receptor. *Eur J Neurosci* **10**: 3721–3736.

Bhattacharjee AK, Chang L, Lee HJ, Bazinet RP, Seemann R, Rapoport SI (2005). D_2 but not D_1 dopamine receptor stimulation augments brain signaling involving arachidonic acid in unanesthetized rats. *Psychopharmacology (Berlin)* **180**: 735–742.

Bland M (2000). *An Introduction to Medical Statistics*, 3rd edn. Oxford University Press: Oxford.

Bosetti F, Rintala J, Seemann R, Rosenberger TA, Contreras MA, Rapoport SI *et al* (2002a). Chronic lithium downregulates cyclooxygenase-2 activity and prostaglandin E_2 concentration in rat brain. *Mol Psychiatr* **7**: 844–850.

Bosetti F, Seemann R, Bell JM, Zahorchak R, Friedman E, Rapoport SI *et al* (2002b). Analysis of gene expression with cDNA microarrays in rat brain after 7 and 42 days of oral lithium administration. *Brain Res Bull* **57**: 205–209.

Braitenberg V, Schüz A (1998). *Cortex: Statistics and Geometry of Neuronal Connectivity*. Springer: Berlin.

Brodal A (1981). *Neurological Anatomy in Relation to Clinical Medicine* 3rd edn Oxford University Press: Oxford.

Browne SE, Muir JL, Robbins TW, Page KJ, Everitt BJ, McCulloch J (1998). The cerebral metabolic effects of manipulating glutamatergic systems within the basal forebrain in conscious rats. *Eur J Neurosci* **10**: 649–663.

Bunney WEJ, Garland-Bunney BL (1987). Mechanisms of action of lithium in affective illness: Basic and clinical implications. In: Meltzer HY (ed). *Psychopharmacology: The Third Generation of Progress*. Raven: New York. pp 553–565.

Bymaster FP, Felder CC (2002). Role of the cholinergic muscarinic system in bipolar disorder and related mechanism of action of antipsychotic agents. *Mol Psychiatr* **7**(Suppl 1): S57–S63.

Cade JF (1999). John Frederick Joseph Cade: Family memories on the occasion of the 50th anniversary of his discovery of the use of lithium in mania. 1949. *Aust NZ J Psychiatr* **33**: 615–622.

Calabrese JR, Bowden C, Woyshville MJ (1995). Lithium and the anticonvulsants in the treatment of bipolar disorder. In: Bloom FE, Kupfer DJ (eds). *Psychopharmacology: The Fourth Generation of Progress*. Raven: New York. pp 1099–1111.

Cepeda C, Levine MS (1998). Dopamine and *N*-methyl-D-aspartate receptor interactions in the neostriatum. *Dev Neurosci* **20**: 1–18.
Chang MC, Bell JM, Purdon AD, Chikhale EG, Grange E (1999). Dynamics of docosahexaenoic acid metabolism in the central nervous system: lack of effect of chronic lithium treatment. *Neurochem Res* **24**: 399–406.
Chang MC, Jones CR (1998). Chronic lithium treatment decreases brain phospholipase A_2 activity. *Neurochem Res* **23**: 887–892.
Chang MCJ, Arai T, Freed LM, Wakabayashi S, Channing MA, Dunn BB *et al* (1997). Brain incorporation of [1-^{11}C]-arachidonate in normocapnic and hypercapnic monkeys, measured with positron emission tomography. *Brain Res* **755**: 74–83.
Chang MCJ, Grange E, Rabin O, Bell JM, Allen DD, Rapoport SI (1996). Lithium decreases turnover of arachidonate in several brain phospholipids. *Neurosci Lett* **220**: 171–174.
Chase TN (2004). Striatal plasticity and extrapyramidal motor dysfunction. *Parkinsonism Relat Disord* **10**: 305–313.
Clark JD, Schievella AR, Nalefski EA, Lin LL (1995). Cytosolic phospholipase A_2. *J Lipid Mediat Cell Signal* **12**: 83–117.
Colbran RJ (2004). Targeting of calcium/calmodulin-dependent protein kinase II. *Biochem J* **378**: 1–16.
Coyle JT, Puttfarcken P (1993). Oxidative stress, glutamate, and neurodegenerative disorders. *Science* **262**: 689–695.
Dai H, Gebhardt K, Carey RJ (1995). Time course effects of MK-801: the relationship between brain neurochemistry and behavior. *Brain Res Bull* **36**: 175–180.
DeGeorge JJ, Nariai T, Yamazaki S, Williams WM, Rapoport SI (1991). Arecoline-stimulated brain incorporation of intravenously administered fatty acids in unanesthetized rats. *J Neurochem* **56**: 352–355.
DeGeorge JJ, Noronha JG, Bell J, Robinson P, Rapoport SI (1989). Intravenous injection of [1-^{14}C]arachidonate to examine regional brain lipid metabolism in unanesthetized rats. *J Neurosci Res* **24**: 413–423.
DeMar Jr JC, Ma K, Bell JM, Rapoport SI (2004a). Half-lives of docosahexaenoic acid in rat brain phospholipids are prolonged by 15 weeks of nutritional deprivation of n-3 polyunsaturated fatty acids. *J Neurochem* **91**: 1125–1137.
DeMar JC, Ma K, Bell J, Rapoport SI (2004b). Brain conversion of linoleic to arachidonic acid is not a major source of the arachidonate found in brain phospholipids of adult rats. *Abstr. International Workshop on Brain Uptake and Utilization of Fatty Acids, Lipids & Lipoproteins, October 7–9, 2004, Bethesda, MD*.
Dennis EA (1994). Diversity of group types, regulation, and function of phospholipase A_2. *J Biol Chem* **269**: 13057–13060.
Du J, Gray NA, Falke CA, Chen W, Yuan P, Szabo ST *et al* (2004). Modulation of synaptic plasticity by antimanic agents: the role of AMPA glutamate receptor subunit 1 synaptic expression. *J Neurosci* **24**: 6578–6589.
Dumuis A, Sebben M, Haynes L, Pin JP, Bockaert J (1988). NMDA receptors activate the arachidonic acid cascade system in striatal neurons. *Nature* **336**: 68–70.
Einat H, Manji HK, Gould TD, Du J, Chen G (2003). Possible involvement of the ERK signaling cascade in bipolar disorder: behavioral leads from the study of mutant mice. *Drug News Perspect* **16**: 453–463.
Farber NB, Jiang XP, Heinkel C, Nemmers B (2002). Antiepileptic drugs and agents that inhibit voltage-gated sodium channels prevent NMDA antagonist neurotoxicity. *Mol Psychiatr* **7**: 726–733.
Farooqui AA, Yang HC, Horrocks L (1997). Involvement of phospholipase A_2 in neurodegeneration. *Neurochem Int* **30**: 517–522.
Feinberg I, Campbell IG, Marrs JC (1995). Intraperitoneal dizocilpine induces cortical spike-wave seizure discharges in rats. *Neurosci Lett* **196**: 157–160.
Felder CC, Dieter P, Kinsella J, Tamura K, Kanterman RY, Axelrod J (1990). A transfected m5 muscarinic acetylcholine receptor stimulates phospholipase A_2 by inducing both calcium influx and activation of protein kinase C. *J Pharmacol Exp Ther* **255**: 1140–1147.
Fenwick PB, Robertson R (1983). Changes in the visual evoked potential to pattern reversal with lithium medication. *Electroencephalogr Clin Neurophysiol* **55**: 538–545.
Ferretti V, Florian C, Costantini VJ, Roullet P, Rinaldi A, De Leonibus E *et al* (2005). Co-activation of glutamate and dopamine receptors within the nucleus accumbens is required for spatial memory consolidation in mice. *Psychopharmacology (Berlin)* **179**: 108–116.
Fisher G, Pelonero AL, Ferguson C (1991). Mania precipitated by prednisone and bromocriptine. *Gen Hosp Psychiatr* **13**: 345–346.
Folch J, Lees M, Sloane Stanley GH (1957). A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* **226**: 497–509.
Fonnum F (1984). Glutamate: a neurotransmitter in mammalian brain. *J Neurochem* **42**: 1–11.
Fukunaga K, Soderling TR, Miyamoto E (1992). Activation of Ca^{2+}/calmodulin-dependent protein kinase II and protein kinase C by glutamate in cultured rat hippocampal neurons. *J Biol Chem* **267**: 22527–22533.
Gould TD, Manji HK (2005). DARPP-32: a molecular switch at the nexus of reward pathway plasticity. *Proc Natl Acad Sci USA* **102**: 253–254.
Gray NA, Du J, Falke CS, Yuan P, Manji HK (2003). Lithium regulates total and synaptic expression of the AMPA glutamate receptor GluR2 *in vitro* and *in vivo*. *Ann NY Acad Sci* **1003**: 402–404.
Guitart X, Nestler EJ (1992). Chronic administration of lithium or other antidepressants increases levels of DARPP-32 in rat frontal cortex. *J Neurochem* **59**: 1164–1167.
Gurd JW, Bissoon N (1997). The *N*-methyl-D-aspartate receptor subunits NR2A and NR2B bind to the SH2 domains of phospholipase C-gamma. *J Neurochem* **69**: 623–630.
Hashimoto R, Fujimaki K, Jeong MR, Christ L, Chuang D (2003). Lithium-induced inhibition of Src tyrosine kinase in rat cerebral cortical neurons: a role in neuroprotection against *N*-methyl-D-aspartate receptor-mediated excitotoxicity. *FEBS Lett* **538**: 145–148.
Hashimoto R, Hough C, Nakazawa T, Yamamoto T, Chuang DM (2002). Lithium protection against glutamate excitotoxicity in rat cerebral cortical neurons: involvement of NMDA receptor inhibition possibly by decreasing NR2B tyrosine phosphorylation. *J Neurochem* **80**: 589–597.
Hayakawa T, Chang MC, Rapoport SI, Appel NM (2001). Selective dopamine receptor stimulation differentially affects [3H]arachidonic acid incorporation, a surrogate marker for phospholipase A_2-mediated neurotransmitter signal transduction, in a rodent model of Parkinson's disease. *J Pharmacol Exp Ther* **296**: 1074–1084.
Hegerl U, Herrmann WM, Ulrich G, Muller-Oerlinghausen B (1990). Effects of lithium on auditory evoked potentials in healthy subjects. *Biol Psychiatr* **27**: 555–560.
Holman RT (1986). Control of polyunsaturated acids in tissue lipids. *J Am Coll Nutr* **5**: 183–211.
Honig A, Arts BM, Ponds RW, Riedel WJ (1999). Lithium induced cognitive side-effects in bipolar disorder: a qualitative analysis and implications for daily practice. *Int Clin Psychopharmacol* **14**: 167–171.
Hough CJ, Irwin RP, Gao XM, Rogawski MA, Chuang DM (1996). Carbamazepine inhibition of *N*-methyl-D-aspartate-evoked calcium influx in rat cerebellar granule cells. *J Pharmacol Exp Ther* **276**: 143–149.
Huo TL, Healy DP (1991). Prostaglandin E_2 production in rat IMCD cells. I. Stimulation by dopamine. *Am J Physiol* **261**: F647–F654.
Ikeda H, Akiyama G, Fujii Y, Minowa R, Koshikawa N, Cools AR (2003). Role of AMPA and NMDA receptors in the nucleus

accumbens shell in turning behaviour of rats: interaction with dopamine receptors. *Neuropharmacology* **44**: 81–87.

Ismailov I, Kalikulov D, Inoue T, Friedlander MJ (2004). The kinetic profile of intracellular calcium predicts long-term potentiation and long-term depression. *J Neurosci* **24**: 9847–9861.

Itokawa M, Yamada K, Iwayama-Shigeno Y, Ishitsuka Y, Detera-Wadleigh S, Yoshikawa T (2003). Genetic analysis of a functional GRIN2A promoter (GT)n repeat in bipolar disorder pedigrees in humans. *Neurosci Lett* **345**: 53–56.

Jarvis MF, Murphy DE, Williams M (1987). Quantitative autoradiographic localization of NMDA receptors in rat brain using [3H]CPP: comparison with [3H]TCP binding sites. *Eur J Pharmacol* **141**: 149–152.

Karkanias NB, Papke RL (1999). Subtype-specific effects of lithium on glutamate receptor function. *J Neurophysiol* **81**: 1506–1512.

Kim DK, Rordorf G, Nemenoff RA, Koroshetz WJ, Bonventre JV (1995). Glutamate stably enhances the activity of two cytosolic forms of phospholipase A_2 in brain cortical cultures. *Biochem J* **310**(Part 1): 83–90.

Kishimoto K, Matsumura K, Kataoka Y, Morii H, Watanabe Y (1999). Localization of cytosolic phospholipase A_2 messenger RNA mainly in neurons in the rat brain. *Neuroscience* **92**: 1061–1077.

Kolko M, Bruhn T, Christensen T, Lazdunski M, Lambeau G, Bazan NG *et al* (1999). Secretory phospholipase A_2 potentiates glutamate-induced rat striatal neuronal cell death *in vivo*. *Neurosci Lett* **274**: 167–170.

Kolko M, Rodriguez de Turco EB, Diemer NH, Bazan NG (2003). Neuronal damage by secretory phospholipase A_2: modulation by cytosolic phospholipase A_2, platelet-activating factor, and cyclooxygenase-2 in neuronal cells in culture. *Neurosci Lett* **338**: 164–168.

Krystal JH, Sanacora G, Blumberg H, Anand A, Charney DS, Marek G *et al* (2002). Glutamate and GABA systems as targets for novel antidepressant and mood-stabilizing treatments. *Mol Psychiatry* **7**(Suppl 1): S71–80.

Kubota T, Jibiki I, Fukushima T, Kurokawa K, Yamaguchi N (1994). Carbamazepine-induced blockade of induction of long-term potentiation in the perforant path-dentate gyrus pathway in chronically prepared rabbits. *Neurosci Lett* **170**: 171–174.

Law AJ, Deakin JF (2001). Asymmetrical reductions of hippocampal NMDAR1 glutamate receptor mRNA in the psychoses. *NeuroReport* **12**: 2971–2974.

Lazarewicz JW, Wroblewski JT, Costa E (1990). *N*-methyl-D-aspartate-sensitive glutamate receptors induce calcium-mediated arachidonic acid release in primary cultures of cerebellar granule cells. *J Neurochem* **55**: 1875–1881.

Lewis SJ, Barres C, Jacob HJ, Ohta H, Brody MJ (1989). Cardiovascular effects of the *N*-methyl-D-aspartate receptor antagonist MK-801 in conscious rats. *Hypertension* **13**: 759–765.

Ma J, Zhang GY (2003). Lithium reduced *N*-methyl-D-aspartate receptor subunit 2A tyrosine phosphorylation and its interactions with Src and Fyn mediated by PSD-95 in rat hippocampus following cerebral ischemia. *Neurosci Lett* **348**: 185–189.

Ma J, Zhang GY, Liu Y, Yan JZ, Hao ZB (2004). Lithium suppressed Tyr-402 phosphorylation of proline-rich tyrosine kinase (Pyk2) and interactions of Pyk2 and PSD-95 with NR2A in rat hippocampus following cerebral ischemia. *Neurosci Res* **49**: 357–362.

Mainen ZF, Malinow R, Svoboda K (1999). Synaptic calcium transients in single spines indicate that NMDA receptors are not saturated. *Nature* **399**: 151–155.

Makrides M, Neumann MA, Byard RW, Simmer K, Gibson RA (1994). Fatty acid composition of brain, retina, and erythrocytes in breast- and formula-fed infants. *Am J Clin Nutr* **60**: 189–194.

Matsuzawa A, Murakami M, Atsumi G, Imai K, Prados P, Inoue K *et al* (1996). Release of secretory phospholipase A_2 from rat neuronal cells and its possible function in the regulation of catecholamine secretion. *Biochem J* **318**(Part 2): 701–709.

McIntyre RS, Mancini DA, Lin P, Jordan J (2004). Treating bipolar disorder. Evidence-based guidelines for family medicine. *Can Fam Phys* **50**: 388–394.

Meador-Woodruff JH, Hogg Jr AJ, Smith RE (2001). Striatal ionotropic glutamate receptor expression in schizophrenia, bipolar disorder, and major depressive disorder. *Brain Res Bull* **55**: 631–640.

Monaghan DT, Cotman CW (1986). Identification and properties of *N*-methyl-D-aspartate receptors in rat brain synaptic plasma membranes. *Proc Natl Acad Sci USA* **83**: 7532–7536.

Moore GJ, Bebchuk JM, Wilds IB, Chen G, Menji HK (2000). Lithium-induced increase in human brain grey matter. *Lancet* **356**: 1241–1242.

Mueller HT, Meador-Woodruff JH (2004). NR3A NMDA receptor subunit mRNA expression in schizophrenia, depression and bipolar disorder. *Schizophr Res* **71**: 361–370.

Mundo E, Tharmalingham S, Neves-Pereira M, Dalton EJ, Macciardi F, Parikh SV *et al* (2003). Evidence that the *N*-methyl-D-aspartate subunit 1 receptor gene (GRIN1) confers susceptibility to bipolar disorder. *Mol Psychiatr* **8**: 241–245.

Murata M, Suzuki M, Tanaka K, Tajiri K, Emori K, Kurachi M (2002). *N*-methyl-D-aspartate-R1 receptor antisense oligodeoxynucleotide modulates pre- and postsynaptic expression of D_2 dopamine receptors in the rat. *Neurosci Lett* **335**: 9–12.

Nair VD, Savelli JE, Mishra RK (1998). Modulation of dopamine D_2 receptor expression by an NMDA receptor antagonist in rat brain. *J Mol Neurosci* **11**: 121–126.

Nishizaki T, Nomura T, Matsuoka T, Enikolopov G, Sumikawa K (1999). Arachidonic acid induces a long-lasting facilitation of hippocampal synaptic transmission by modulating PKC activity and nicotinic ACh receptors. *Brain Res Mol Brain Res* **69**: 263–272.

Nonaka S, Chuang DM (1998). Neuroprotective effects of chronic lithium on focal cerebral ischemia in rats. *NeuroReport* **9**: 2081–2084.

Ong WY, Sandhya TL, Horrocks LA, Farooqui AA (1999). Distribution of cytoplasmic phospholipase A_2 in the normal rat brain. *J Hirnforsch* **39**: 391–400.

Ormandy GC, Song L, Jope RS (1991). Analysis of the convulsant-potentiating effects of lithium in rats. *Exp Neurol* **111**: 356–361.

Otmakhov N, Tao-Cheng JH, Carpenter S, Asrican B, Dosemeci A, Reese TS *et al* (2004). Persistent accumulation of calcium/calmodulin-dependent protein kinase II in dendritic spines after induction of NMDA receptor-dependent chemical long-term potentiation. *J Neurosci* **24**: 9324–9331.

Pachet AK, Wisniewski AM (2003). The effects of lithium on cognition: an updated review. *Psychopharmacology (Berlin)* **170**: 225–234.

Pal R, Eaton MJ, Islam S, Hake-Frendscho M, Kumar KN, Michaelis EK (1999). Immunocytochemical and *in situ* hybridization studies of the expression and distribution of three subunits of a complex with *N*-methyl-D-aspartate receptor-like properties. *Neuroscience* **94**: 1291–1311.

Pardue S, Rapoport SI, Bosetti F (2003). Co-localization of cytosolic phospholipase A_2 and cyclooxygenase-2 in Rhesus monkey cerebellum. *Brain Res Mol Brain Res* **116**: 106–114.

Paxinos G, Watson C (1987). *The Rat Brain in Stereotaxic Coordinates*, 3rd edn. Academic Press: New York.

Peet M, Peters S (1995). Drug-induced mania. *Drug Saf* **12**: 146–153.

Pellerin L, Wolfe LS (1991). Release of arachidonic acid by NMDA-receptor activation in the rat hippocampus. *Neurochem Res* **16**: 983–989.

Petralia RS, Yokotani N, Wenthold RJ (1994). Light and electron microscope distribution of the NMDA receptor subunit NMDAR1 in the rat nervous system using a selective anti-peptide antibody. *J Neurosci* **14**: 667–696.

Piomelli D, Di Marzo V (1993). Dopamine D_2 receptor signaling via the arachidonic acid cascade: modulation by cAMP-dependent protein kinase A and prostaglandin E_2. *J Lipid Mediat* **6**: 433–443.

Post RM, Jimerson DC, Bunney Jr WE, Goodwin FK (1980). Dopamine and mania: behavioral and biochemical effects of the dopamine receptor blocker pimozide. *Psychopharmacology (Berlin)* **67**: 297–305.

Qu Y, Chang L, Klaff J, Balbo A, Rapoport SI (2003). Imaging brain phospholipase A_2 activation in awake rat in response to 5-$HT_{2A/2C}$ agonist (+/−)-2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI). *Neuropsychopharmacology* **28**: 244–252.

Raichle ME, Gusnard DA (2002). Appraising the brain's energy budget. *Proc Natl Acad Sci USA* **99**: 10237–10239.

Rao JS, Rapoport SI, Bosetti F (2005). Decrease in the AP-2 DNA binding activity and in the protein expression of AP-2alpha and AP-2beta in frontal cortex of rats treated with lithium for 6 weeks. *Neuropsychopharmacology* **30**: 2006–2013.

Rapoport SI (2001). *In vivo* fatty acid incorporation into brain phospholipids in relation to plasma availability, signal transduction and membrane remodeling. *J Mol Neurosci* **16**: 243–261.

Rapoport SI (2003). *In vivo* approaches to quantifying and imaging brain arachidonic and docosahexaenoic acid metabolism. *J Pediatr* **143**: S26–S34.

Rapoport SI, Bosetti F (2002). Do lithium and anticonvulsants target the brain arachidonic acid cascade in bipolar disorder? *Arch Gen Psychiatr* **59**: 592–596.

Rintala J, Seemann R, Chandrasekaran K, Rosenberger TA, Chang L, Contreras MA *et al* (1999). 85 kDa cytosolic phospholipase A_2 is a target for chronic lithium in rat brain. *NeuroReport* **10**: 3887–3890.

Robinson PJ, Noronha J, DeGeorge JJ, Freed LM, Nariai T, Rapoport SI (1992). A quantitative method for measuring regional *in vivo* fatty-acid incorporation into and turnover within brain phospholipids: Review and critical analysis. *Brain Res Rev* **17**: 187–214.

Rogue P, Zwiller J, Malviya AN, Vincendon G (1990). Phosphorylation by protein kinase C modulates agonist binding to striatal dopamine D_2 receptors. *Biochem Int* **22**: 575–582.

Rothman SM, Olney JW (1995). Excitotoxicity and the NMDA receptor—still lethal after eight years. *Trends Neurosci* **18**: 57–58.

Rowe MK, Chuang DM (2004). Lithium neuroprotection: molecular mechanisms and clinical implications. *Expert Rev Mol Med* **6**: 1–18.

Sanfeliu C, Hunt A, Patel AJ (1990). Exposure to *N*-methyl-D-aspartate increases release of arachidonic acid in primary cultures of rat hippocampal neurons and not in astrocytes. *Brain Res* **526**: 241–248.

Sapirstein A, Bonventre JV (2000). Specific physiological roles of cytosolic phospholipase A_2 as defined by gene knockouts. *Biochim Biophys Acta* **1488**: 139–148.

Scarr E, Pavey G, Sundram S, MacKinnon A, Dean B (2003). Decreased hippocampal NMDA, but not kainate or AMPA receptors in bipolar disorder. *Bipolar Disord* **5**: 257–264.

Schmitt JM, Guire ES, Saneyoshi T, Soderling TR (2005). Calmodulin-dependent kinase kinase/calmodulin kinase I activity gates extracellular-regulated kinase-dependent long-term potentiation. *J Neurosci* **25**: 1281–1290.

Scott DB, Blanpied TA, Ehlers MD (2003). Coordinated PKA and PKC phosphorylation suppresses RXR-mediated ER retention and regulates the surface delivery of NMDA receptors. *Neuropharmacology* **45**: 755–767.

Shibata S, Tominaga K, Hamada T, Watanabe S (1992). Excitatory effect of *N*-methyl-D-aspartate and kainate receptor on the 2-deoxyglucose uptake in the rat suprachiasmatic nucleus *in vitro*. *Neurosci Lett* **139**: 83–86.

Shimizu T, Wolfe LS (1990). Arachidonic acid cascade and signal transduction. *J Neurochem* **55**: 1–15.

Sokoloff L (1999). Energetics of functional activation in neural tissues. *Neurochem Res* **24**: 321–329.

Stewart CA, Reid IC (2002). Antidepressant mechanisms: functional and molecular correlates of excitatory amino acid neurotransmission. *Mol Psychiatr* **7**(Suppl 1): S15–S22.

Stip E, Dufresne J, Lussier I, Yatham L (2000). A double-blind, placebo-controlled study of the effects of lithium on cognition in healthy subjects: mild and selective effects on learning. *J Affect Disord* **60**: 147–157.

Strokin M, Sergeeva M, Reiser G (2004). Role of Ca^{2+}-independent phospholipase A_2 and n-3 polyunsaturated fatty acid docosahexaenoic acid in prostanoid production in brain: perspectives for protection in neuroinflammation. *Int J Dev Neurosci* **22**: 551–557.

Sultzer DL, Cummings JL (1989). Drug-induced mania—causative agents, clinical characteristics and management. A retrospective analysis of the literature. *Med Toxicol Adverse Drug Exp* **4**: 127–143.

Tabachnick BG, Fidell LS (2001). *Computer Assisted Research Design and Analysis*. Allyn and Bacon: Boston, MA.

Takeuchi Y, Miyamoto E, Fukunaga K (2002). Activation of the rat dopamine D_2 receptor promoter by mitogen-activated protein kinase and Ca^{2+}/calmodulin-dependent protein kinase II pathways. *J Neurochem* **83**: 784–796.

Takita M, Yokoi H, Mizuno T (1997). NMDA receptor clustering in rat prefrontal cortex revealed by *in vitro* calcium macroimaging. *NeuroReport* **8**: 551–553.

Tapia-Arancibia L, Rage F, Récasens M, Pin JP (1992). NMDA receptor activation stimulates phospholipase A_2 and somatostatin release from rat cortical neurons in primary cultures. *Eur J Pharmacol* **225**: 253–262.

Tencé M, Cordier J, Premont J, Glowinski J (1994). Muscarinic cholinergic agonists stimulate arachidonic acid release from mouse striatal neurons in primary culture. *J Pharmacol Exp Ther* **269**: 646–653.

Tseng KY, O'Donnell P (2004). Dopamine-glutamate interactions controlling prefrontal cortical pyramidal cell excitability involve multiple signaling mechanisms. *J Neurosci* **24**: 5131–5139.

Ulrich G, Herrmann WM, Hegerl U, Muller-Oerlinghausen B (1990). Effect of lithium on the dynamics of electroencephalographic vigilance in healthy subjects. *J Affect Disord* **20**: 19–25.

Valjent E, Pascoli V, Svenningsson P, Paul S, Enslen H, Corvol JC *et al* (2005). Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. *Proc Natl Acad Sci USA* **102**: 491–496.

Vial D, Piomelli D (1995). Dopamine D_2 receptors potentiate arachidonate release via activation of cytosolic, arachidonic-specific phospholipase A_2. *J Neurochem* **64**: 2765–2772.

Wang HY, Johnson GP, Friedman E (2001). Lithium treatment inhibits protein kinase C translocation in rat brain cortex. *Psychopharmacology (Berlin)* **158**: 80–86.

Wang X, Zhong P, Gu Z, Yan Z (2003). Regulation of NMDA receptors by dopamine D_4 signaling in prefrontal cortex. *J Neurosci* **23**: 9852–9861.

Washizaki K, Smith QR, Rapoport SI, Purdon AD (1994). Brain arachidonic acid incorporation and precursor pool specific activity during intravenous infusion of unesterified [3H]arachidonate in the anesthetized rat. *J Neurochem* **63**: 727–736.

Weerasinghe GR, Rapoport SI, Bosetti F (2004). The effect of chronic lithium on arachidonic acid release and metabolism in rat brain does not involve secretory phospholipase A_2 or lipoxygenase/cytochrome P450 pathways. *Brain Res Bull* **63**: 485–489.

Wei S, Ong WY, Thwin MM, Fong CW, Farooqui AA, Gopalakrishnakone P *et al* (2003). Group IIA secretory phospholipase A_2 stimulates exocytosis and neurotransmitter release in

pheochromocytoma-12 cells and cultured rat hippocampal neurons. *Neuroscience* **121**: 891–898.

Weichel O, Hilgert M, Chatterjee SS, Lehr M, Klein J (1999). Bilobalide, a constituent of Ginkgo biloba, inhibits NMDA-induced phospholipase A_2 activation and phospholipid breakdown in rat hippocampus. *Naunyn Schmiedebergs Arch Pharmacol* **360**: 609–615.

Wenthold RJ, Prybylowski K, Standley S, Sans N, Petralia RS (2003). Trafficking of NMDA receptors. *Annu Rev Pharmacol Toxicol* **43**: 335–358.

Williams JH, Errington ML, Lynch MA, Bliss TV (1989). Arachidonic acid induces a long-term activity-dependent enhancement of synaptic transmission in the hippocampus. *Nature* **341**: 739–742.

Wong EH, Kemp JA, Priestley T, Knight AR, Woodruff GN, Iversen LL (1986). The anticonvulsant MK-801 is a potent *N*-methyl-D-aspartate antagonist. *Proc Natl Acad Sci USA* **83**: 7104–7108.

Wong EH, Knight AR, Woodruff GN (1988). [3H]MK-801 labels a site on the *N*-methyl-D-aspartate receptor channel complex in rat brain membranes. *J Neurochem* **50**: 274–281.

Yang HC, Mosior M, Johnson CA, Chen Y, Dennis EA (1999). Group-specific assays that distinguish between the four major types of mammalian phospholipase A_2. *Anal Biochem* **269**: 278–288.

Yegin A, Akbas SH, Ozben T, Korgun DK (2002). Secretory phospholipase A_2 and phospholipids in neural membranes in an experimental epilepsy model. *Acta Neurol Scand* **106**: 258–262.

Zarate Jr CA, Du J, Quiroz J, Gray NA, Denicoff KD, Singh J *et al* (2003). Regulation of cellular plasticity cascades in the pathophysiology and treatment of mood disorders: role of the glutamatergic system. *Ann NY Acad Sci* **1003**: 273–291.

Zeise ML, Kasparow S, Zieglgansberger W (1991). Valproate suppresses *N*-methyl-D-aspartate-evoked, transient depolarizations in the rat neocortex *in vitro*. *Brain Res* **544**: 345–348.